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Description of two new species of *Dicranomyia* (*Erostrata*) crane fly (Diptera, Limoniidae) from Korea, with remarks on DNA barcoding and updated taxonomic key

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Abstract

Two new crane fly species, *Dicranomyia* (*Erostrata*) *jejuensis* **sp. nov.** and *D.* (*E.*) *koreana* **sp. nov.**, from Korea are described on the basis of morphology and mitochondrial *COI* sequences. DNA barcode sequences for other four *D.* (*Erostrata*) species from Korea are also provided for the first time. The identification key for all known *D.* (*Erostrata*) species is presented.

Keywords

Dicranomyia (*Erostrata*) *jejuensis*, *Dicranomyia* (*Erostrata*) *koreana*, DNA barcode, Limoniinae, taxonomy

Introduction

Genus *Dicranomyia* Stephens, 1829, is the largest genus of the Limoniidae and, as such, contains 1,136 species and 24 subgenera, including the subgenus *D.* (*Erostrata*) Savchenko, 1976. Twelve species of this subgenus have been reported from the Palearctic, Nearctic, and Oriental regions (Oosterbroek 2023). Adult crane flies are typically found in moist deciduous forests, shrubs along small streams, and abandoned

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farmlands near streams (Alexander 1931a, b; Podenas et al. 2019, 2020). Meanwhile, larval *D.* (*E.*) *globithorax* has been reported in fungus on decaying logs (Rogers 1930). Data on DNA barcoding for this subgenus is unknown so far.

Alexander (1934) first reported *D.* (*E.*) *tabashii* (as *Limonia* (*Limonia*) *tabashii*) from Suigen (= Suwon) in Korea, and Podenas et al. (2019, 2020) added three and one additional species, respectively, to the recognized fauna of Korea. In Japan, Kato et al. (2018) revised six *Dicranomyia* species of the subgenus *Erostrata* including three new species and suggested that *Limonia congesta* Alexander, 1976 and *Limonia striopleura* Edwards, 1919 be classified as members of subgenus *Erostrata* based on non-genitalic characters.

Here, two new *D.* (*Erostrata*) crane fly species are described from Korea, providing identification for all Korean members of the subgenus. A DNA barcode (*COI*) dataset for six *Dicranomyia* species of the subgenus *Erostrata* from Korea is also presented for the first time.

Materials and methods

Crane fly sampling and examination

Crane fly adults were collected using insect nets or Malaise traps and preserved in 80% ethanol (Table 1). Wings and legs of selected adults were slide mounted using Euparal. Meanwhile, the genitalia and ovipositors of male and female specimens, respectively, were cleared overnight using 10% KOH and then preserved in micro-vials with glycerol. Specimens were examined using a microscope Olympus SZ51 with a digital camera Canon EOS 6D (Tokyo, Japan) and Olympus BX53 with camera Nikon Z7 (Tokyo, Japan).

The terminologies used to describe the morphology generally follow Cumming and Wood (2017), and de Jong (2017) for wing venation. The species distribution is given according to Oosterbroek (2023).

Specimen depositories are as follows: **KUEM** – Korea University Entomological Museum, Seoul, Republic of Korea; **NIBR** – National Institute of Biological Resources, Incheon, Republic of Korea.

DNA extraction and sequence generation

Total genomic DNA was extracted from the leg muscle of using the DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. *COI* sequences were amplified and sequenced following Suh et al. (2019), except for the use of primers LCO1490 (5'-GGT CAA CAA ATC ATA AAG ATA TTG G-3'; Folmer et al. 1994) and C1-N-2191 (5'-CCC GGT AAA ATT AAA ATA TAA ACT TC-3'; Simon et al. 1994), which targeted a 676-bp region of *COI*. All sequences were submitted to GenBank (accession numbers: OM102975–OM102983; OP081140; OP093621).

Table 1. Collection data of the Korean *Dicranomyia* (*Erostrata*) species used in the barcode analyses of this study.

Species*	GenBank accession number	Specimen code	Locality	Date	Collector(s)	Coordinates
D. (*E.*) *globithorax*	OM102980	CF21-0149	Gangwon-do, Wonju-si	3 Sep. 2021	J. Kim, D. Lee	37°29'50.88"N, 130°53'23.78"E
D. (*E.*) *jejuensis* sp. nov.	OM102981	CF21-0150H	Jeju-do, Seoguipo-si	14 Jun.–4 Aug. 2021	Y. J. Bae	33°19'49.07"N, 126°37'28.08"E
	OM102982	CF21-0150P	Jeju-do, Seoguipo-si	4 Aug.–8 Sep. 2021	Y. J. Bae	33°19'49.07"N, 126°37'28.08"E
	OM102983	CF21-0151	Jeju-do, Seoguipo-si	4 Aug.–8 Sep. 2021	Y. J. Bae	33°19'49.07"N, 126°37'28.08"E
D. (*E.*) *koreana* sp. nov.	OM102979	CF21-0148	Jeju-do, Seoguipo-si	13 Jun.–4 Aug. 2021	Y. J. Bae	33°20'57.10"N, 126°29'43.29"E
	OP081140	CF21-0152	Gyeongsangnam-do, Sancheong-gun	28 Jul. 2021	J. Kim, C. Lim, D. Lee, W. Lee	35°18'37.83"N, 127°45'05.47"E
D. (*E.*) *submelas*	OM102978	CF21-0133	Jeju-do, Seoguipo-si	4 Aug.–8 Sep. 2021	Y. J. Bae	33°19'49.07"N, 126°37'28.08"E
D. (*E.*) *tabashii*	OM102975	CF21-0115	Gyeongsangnam-do, Sancheong-gun	28 Jul. 2021	J. Kim, C. Lim, D. Lee, W. Lee	35°18'37.83"N, 127°45'05.47"E
	OM102976	CF21-0115f	Gyeongsangnam-do, Sancheong-gun	28 Jul. 2021	J. Kim, C. Lim, D. Lee, W. Lee	35°18'37.83"N, 127°45'05.47"E
D. (*E.*) *yazuensis*	OM102977	CF21-0132	Gangwon-do, Pyeongchang-gun	28 Jul.–15 Sep. 2020	Y. J. Bae	37°47'05.67"N, 128°34'16.97"E
D. (*D.*) *kandybinae*	OP093621	CF21-0099	Gangwon-do, Wonju-si	23 Jul.–3 Sep. 2021	Y. J. Bae	37°17'26.50"N, 128°04'54.77"E

*Specimens were morphologically identified by J. Kim.

DNA barcode sequence analysis

DNA barcode analysis was performed using 11 *COI* sequences (Table 1), which were generated from six Korean *D.* (*Erostrata*) species (10 sequences), and the outgroup species *D.* (*Dicranomyia*) *kandybinae* Savchenko, 1987 (1 sequence). Phylogenetic analyses were conducted using the neighbor-joining (NJ) method and Kimura-2-parameter model (Kimura 1980), with 1,000 bootstrap replicates, in MEGA X (Kumar et al. 2018). Sequence divergence was estimated via pairwise comparison of the uncorrected genetic distances (*p*-distances) in MEGA X, using the complete deletion option.

Checklist of the world *Dicranomyia* (*Erostrata*) crane flies (Oosterbroek, 2023)

Dicranomyia (*Erostrata*) *canis* (Alexander, 1931b)
Dicranomyia (*Erostrata*) *cnephosa* (Alexander, 1959)
Dicranomyia (*Erostrata*) *congesta* (Alexander, 1967)
Dicranomyia (*Erostrata*) *cynotis* (Alexander, 1931a)
Dicranomyia (*Erostrata*) *globithorax* Osten Sacken, 1869
Dicranomyia (*Erostrata*) *globulithorax* Alexander, 1924
Dicranomyia (*Erostrata*) *jejuensis* sp. nov.
Dicranomyia (*Erostrata*) *koreana* sp. nov.
Dicranomyia (*Erostrata*) *melas* (Alexander, 1934)

Dicranomyia (*Erostrata*) *reniformis* Kato, Tachi & Gelhaus, 2018
Dicranomyia (*Erostrata*) *striopleura* (Edwards, 1919)
Dicranomyia (*Erostrata*) *submelas* Kato, Tachi & Gelhaus, 2018
Dicranomyia (*Erostrata*) *tabashii* (Alexander, 1934)
Dicranomyia (*Erostrata*) *yazuensis* Kato, Tachi & Gelhaus, 2018

Key to the species of *Dicranomyia* (*Erostrata*), updated from Kato et al. (2018) and Podenas et al. (2020)

1 Tarsi with white bands ..... ***Dicranomyia* (*Erostrata*) *congesta* (India)**
– Tarsi without bands **2**
2 Pleuron with broad, blackish brown lateral stripe ..... ***Dicranomyia* (*Erostrata*) *striopleura* (Indonesia, Malaysia)**
– Pleuron without lateral stripe **3**
3 Scutellum obscure yellow **4**
– Scutellum yellowish brown to blackish brown **6**
4 Rostrum black. Wing strongly blackened ..... ***Dicranomyia* (*Erostrata*) *cnephosa* (Nepal)**
– Rostrum pale. Wing tinged with pale brown **5**
5 Palpus 2-segmented. Male seventh sternite with strongly darkened internal sac ..... ***Dicranomyia* (*Erostrata*) *tabashii* (Japan, Korea, Russia)**
– Palpus 3-segmented. Male seventh sternite with slightly darkened internal sac with rounded entrance ... ***Dicranomyia* (*Erostrata*) *koreana* sp.nov. (Korea)**
6 Gonostylus with black spines on mesal face **7**
– Gonostylus without black spines **12**
7 Gonostylus narrowed to a point, triangular **8**
– Gonostylus not as above **9**
8 Mesal face of gonostylus densely covered with black setae ..... ***Dicranomyia* (*Erostrata*) *cynotis* (Philippines)**
– Mesal face of gonostylus with black setae restricted to distal 1/2 ..... ***Dicranomyia* (*Erostrata*) *canis* (Philippines)**
9 Paramere distally with rounded tip **10**
– Paramere distally with pointed tip **11**
10 Gonostylus elongate with truncated apex ..... ***Dicranomyia* (*Erostrata*) *globithorax* (Canada, Japan, Korea, USA)**
– Gonostylus elongate with angled apex, length of angled apex ca 1/5 of gonostylus ..... ***Dicranomyia* (*Erostrata*) *globulithorax* (Japan, Korea, Russia)**
11 Gonocoxite with apically rounded ventromesal lobe. Gonostylus shallowly concaved at inner apical edge, the area 1/5 as long as gonostylus ..... ***Dicranomyia* (*Erostrata*) *melas* (Taiwan)**
– Gonocoxite with apically truncated ventromesal lobe. Gonostylus deeply emarginated at inner apical edge, the area 1/3 as long as gonostylus ..... ***Dicranomyia* (*Erostrata*) *submelas* (Japan, Korea)**

12 Gonostylus stout and reniform, tip of ventral surface covered with dense, fine setae..... ***Dicranomyia* (*Erostrata*) *reniformis* (Japan)**
– Gonostylus not reniform, tip of ventral surface without dense, fine setae.... **13**
13 Palpus 2-segmented. Gonostylus tapered in distal 1/3, without apical spine.. ..... ***Dicranomyia* (*Erostrata*) *yazuensis* (Japan, Korea)**
– Palpus 3-segmented. Gonostylus tapered in distal 1/2, with black apical spine..... ***Dicranomyia* (*Erostrata*) *jejuensis* sp. nov. (Korea)**

Taxonomic accounts

Family Limoniidae Speiser, 1909
Subfamily Limoniinae Speiser, 1909
Genus *Dicranomyia* Stephens, 1829

Subgenus *Erostrata* Savchenko, 1976

Dicranomyia (*Erostrata*) Savchenko in Savchenko and Krivolutskaya 1976: 131–132; Kato et al. 2018: 182; Podenas et al. 2019: 72–73.

Type species. *Dicranomyia globithorax* Osten Sacken, 1869 (original designation).

Diagnosis. Rostrum is very short or reduced. Number of palpomeres ranges from one to three. Wings have no patterns, even in stigmal region. Third and fourth tarsomere are slightly swollen. Internal sac or notch is located on the male seventh sternite. Gonocoxite has ventromesal lobe. Gonostylus is one paired, with one or two setae arising from small tubercle on outer surface.

***Dicranomyia* (*Erostrata*) *jejuensis* sp. nov.**
https://zoobank.org/9659D5F8-C578-4FAA-B16A-6AF8A9AE39E5
Figs 1, 2

Type material. ***Holotype*:** Korea • ♂; Jeju-do, Seogwipo-si, Namwon-eup, Sillye-ri, Iseungi-oreum Volcanic Cone; 33°20.24'N, 126°37.25'E; alt. 450 m; 4 Aug.–8 Sep. 2021; Y. J. Bae leg.; Malaise trap; GenBank: OM102981; CF21-0150H; NIBR.

***Paratypes*:** Korea • 1 ♀; same data as holotype, 14 Jul.–4 Aug. 2021; GenBank: OM102983; CF21-0151; KUEM • 1 ♂; same data as holotype; GenBank: OM102982; CF21-0150P; KUEM.

Diagnosis. Palpus is 3-segmented. Male seventh sternite has shallow V-shaped notch. Outer face of gonostylus has single seta arising from tubercle. Distal lobe of paramere has a hooked tip with a subapical process.

Description. Male (holotype). Body length 4.3 mm, wing length 4.6 mm, antenna length 0.9 mm. General body coloration pale yellow to yellowish brown (Fig. 1A).

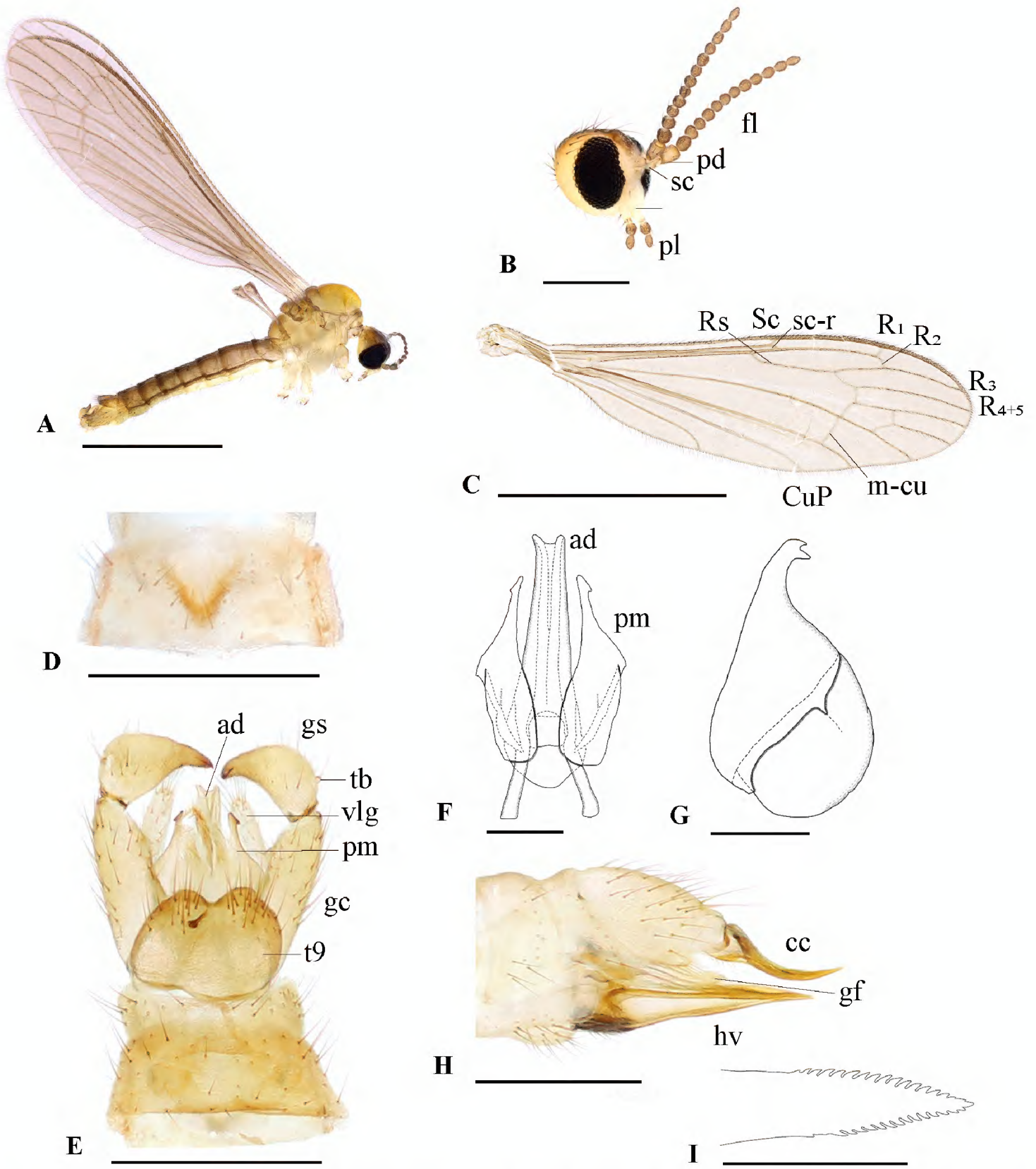

Figure 1. *Dicranomyia* (*Erostrata*) *jejuensis* sp. nov. **A** habitus, male (paratype) **B** head, female (paratype) **C** wing, male (paratype) **D** male seventh sternite, ventral view **E** male terminalia, dorsal view **F** aedeagal complex, dorsal view **G** paramere, lateral view **H** female terminalia, lateral view **I** tip of hypovalva, lateral view. Abbreviations: ad – aedeagus; cc – cercus; fl – flagellum; gc – gonocoxite; gf – genital fork; gs – gonostylus; hv – hypovalva; pd – pedicel; pl – palpus; pm – paramere; sc – scape; tb – tubercle; t9 – ninth tergite; vlg – ventromesal lobe of gonocoxite. Scale bars: 2 mm (**A, C**); 0.5 mm (**B, D, E, H**); 0.1 mm (**F, G, I**).

Head (Fig. 1B). Dark brown dorsally, pale ventrally. Antennae 14-segmented; scape pale yellow; pedicel yellowish brown; flagellum brown. Rostrum pale, rudimentary. Palpus 3-segmented; basal 2/3 of first palpomere pale; remainder of palpus brown.

Figure 2. Habitat of *Dicranomyia* (*Erostrata*) *jejuensis* sp. nov.

Thorax. Prescutum and presutural scutum yellow. Postsutural scutum, scutellum and mediotergite yellowish brown. Pleuron uniformly dull yellow, without lateral stripes. Wing (Fig. 1C) tinged with pale brown; veins brown; Sc ending before middle of Rs; sc-r at tip of Sc; Rs arched at base; R_1 and R_2 nearly transverse, at the same level; R_3 and R_{4+5} parallel to each other; discal medial cell closed; m-cu before fork of M; CuP ending beyond tip of Sc. Halter pale brown. Legs with coxae and trochanters pale; base of femora pale, remainder of femora brown; tibiae and tarsi brown. Femur II 3.3 mm; femur III 3.8 mm; tibia II 3.5 mm; tibia III 4.0 mm; tarsus II 3.2 mm; tarsus III 2.8 mm. Claw without additional tooth.

Abdomen. Tergites yellowish brown, sternites 1–4 yellow, remaining yellowish brown. Seventh sternite with shallow V-shaped notch with darkened margin (Fig. 1D).

Male terminalia (Fig. 1E–G). Yellow. Ninth tergite with posterior margin rounded (Fig. 1E), medially with distinct emargination, distal part covered with setae. Gonocoxite elongated, approximately 3× as long as width at base, with elongated, setose ventromesal lobe. Gonostylus yellowish brown at base, turning dark distally; distal 1/2 of gonostylus gradually tapered toward apex, with short black spine at tip; dorsal margin near the base with small tubercle bearing pale, stout seta. Paramere (Fig. 1F, G) with basal 1/2 pale and apical 1/2 yellowish, distal lobe darkened apically with hooked

tip and subapical projection. Aedeagus (Fig. 1F) as long as gonocoxite, bifid, curved outwards at tip.

Female. Body length 4.5 mm, wing length 4.8 mm, antenna length 0.9 mm ($N = 1$). General body coloration brighter than male. Femur I 2.8 mm; II 3.2 mm; III 3.4 mm; tibia I 3.2 mm; I: 3.1 mm; III 3.4 mm; tarsus I 3.0 mm; II 2.6 mm; III 2.4 mm.

Female terminalia (Fig. 1H–I). Yellow. Cercus curved upwardly (Fig. 1H), wider at base, narrowing towards acute tip. Genital fork long, ca 1.5× as long as width, extending to base of cercus. Hypovalva wedge-shaped, reaching to ca 2/3 of cercus, with distinct black spot at base. Dorsal and ventral margin of hypovalva serrated near tip (Fig. 1I).

Etymology. Specific name "*jejuensis*" refers to the type locality, Jejudo Island, Korea.

Distribution. The species is currently only known from Jejudo Island, Korea.

Habitats. Adults of this species are found in deciduous forests with moss-covered rocks along intermittent, rocky mountain streams (Fig. 2) and co-occur with *D.* (*E.*) *submelas*.

Period of activity. Adults were collected from June through early September.

Remarks. *Dicranomyia* (*E.*) *jejuensis* sp. nov. is morphologically similar to *D.* (*E.*) *yazuensis* based on the male genital structures, but it can be distinguished by the following characters: pleuron entirely dull yellow (vs dark dorsally); palpus 3-segmented (vs 2-segmented); distal 1/2 of gonostylus tapered to tip (vs distal 2/3 strongly narrowed toward tip); posterior margin of male seventh sternite with shallow V-shaped notch (vs long triangular notch); distal part of paramere with hooked tip (vs straight tip).

Dicranomyia (*Erostrata*) *koreana* sp. nov.

https://zoobank.org/163BF75E-826C-4E64-AAE4-CFAA23F2E22D

Figs 3, 4

Type material. ***Holotype*:** Korea • ♂; Jeju-do, Seogwipo-si, Hawon-dong, Mt. Hallasan; 33°20.95'N, 126°29.72'E; alt. 1220 m; 13 Jun.–4 Aug. 2021; Y. J. Bae leg.; Malaise trap; GenBank: OM102979; CF21-0148; NIBR.

***Paratypes*:** Korea • 1 ♂; Gyeonggi-do, Gapyeong-si, Buk-myeon, Jeokmok-ri, Garim-gyo (Br.); 37°58.60'N, 127°26.55'E; alt. 300 m; 25 Jul.–1 Aug. 2015; Y. J. Bae leg.; Malaise trap; published as *D.* (*E.*) *tabashii* by Podenas et al. (2019); KUEM • 2 ♂♂, 1 ♀; same data as for preceding; 2–8 Aug. 2015; published as *D.* (*E.*) *tabashii* by Podenas et al. (2019); KUEM • 1 ♂; same data as for preceding; 23–29 Jul. 2016; published as *D.* (*E.*) *tabashii* by Podenas et al. (2019); KUEM • 1 ♂; Gangwon-do, Inje-gun, Girin-myeon, Bangdong-ri, Mt. Bangtaesan; 37°54.50'N, 128°24.41'E; alt. 690 m; 30 Jul.–16 Sep. 2019; Y. J. Bae leg.; Malaise trap; KUEM • 1 ♂; Gyeonsangnam-do, Sancheong-gun, Sicheon-myeon, Jungsan-ri, Jungsan-ri Campsite, Mount Jirisan; 35°18.63'N, 127°45.09'E; alt. 700 m; 28 Jul. 2021; J. Kim, C. Lim, D. Lee, W. Lee leg.; sweeping; GenBank: OP081140; CF21-0152; KUEM.

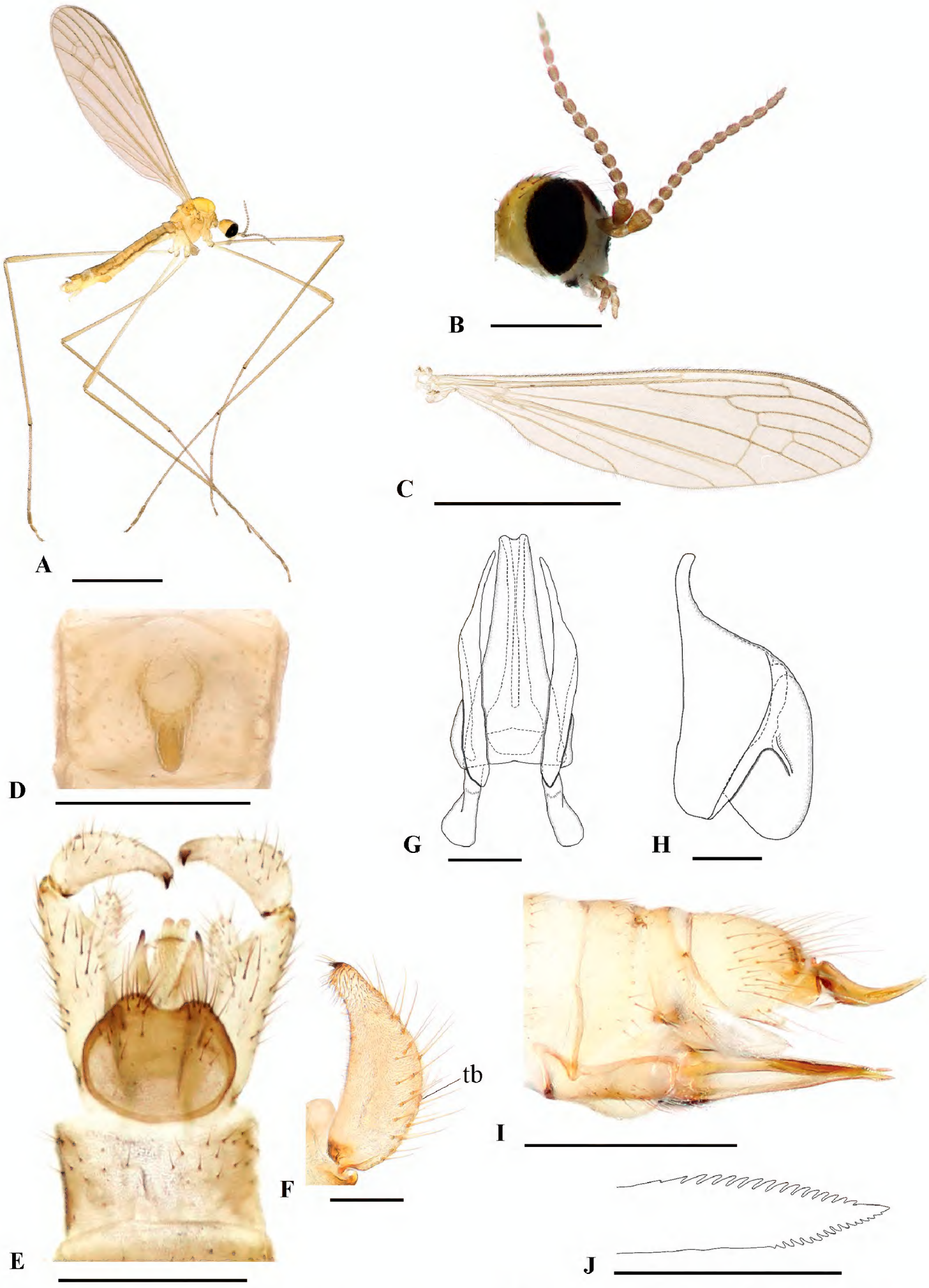

Figure 3. *Dicranomyia* (*Erostrata*) *koreana* sp. nov. **A** habitus, male (holotype) **B** head, male (paratype) **C** wing, male (paratype) **D** male seventh sternite, ventral view **E** male terminalia, dorsal view **F** gonostylus, ventral view **G** aedeagal complex, dorsal view **H** paramere, lateral view **I** female terminalia, lateral view **J** tip of hypovalva, lateral view. Abbreviation: tb – tubercle. Scale bars: 2 mm (**A, C**); 0.5 mm (**B, D, E, I**); 0.1 mm (**F, G, H, J**).

Diagnosis. Palpus is 3-segmented. Center of male seventh sternite has a deep conical internal sac that has a wide, round entrance. Outer face of gonostylus has two setae arising from a small tubercle. Paramere is elongated and narrow, distally with a darkened tip.

Description. Male (holotype). Body length 3.5 mm, wing length 4.5 mm, antenna length 0.7 mm. General body coloration yellow (Fig. 3A).

Head (Fig. 3B). Dark brown dorsally, yellow ventrally. Vertex with a distinct black spot between compound eyes. Antennae 14-segmented; scape pale brown; pedicel yellowish brown; flagellum brown. Rostrum pale, reduced. Palpus 3-segmented, yellowish brown; basal 1/2 of first palpomere pale.

Thorax. Prescutum, scutum and scutellum yellow. Mediotergite yellowish brown. Pleuron entirely pale yellow, without lateral stripes. Wing (Fig. 3C) tinged with pale brown; veins brown; tip of Sc reaching ca 1/3 of Rs; sc-r at tip of Sc; R_1 indistinct; R_2 ending distinctly beyond tip of R_1; discal cell closed; m-cu slightly beyond fork of M. Halter pale brown. Legs with coxae and trochanters pale yellow; femora and tibiae brownish yellow; tarsal segments light brown. Femur I 2.4 mm; femur II 2.8 mm; femur III 3.1 mm; tibia I 2.8 mm; tibia II 2.4 mm; tibia III 2.8 mm; tarsus I 2.9 mm; tarsus II 2.5 mm; tarsus III 2.4 mm. Claw without additional tooth.

Abdomen. Tergites yellow except pale eighth tergite; sternites paler. Seventh sternite (Fig. 3D) with central deep, conical, slightly darkened sac, and rounded entrance.

Male terminalia (Fig. 3E–H). Yellow. Ninth tergite rounded (Fig. 3E), wider basally, narrower apically; posterior margin with two short, setose lateral lobes separated by shallow U-shaped incision. Gonocoxite elongated, ventromesal lobe margin rounded and covered with setae, reaching beyond tip of aedeagus. Gonostylus (Fig. 3E, F) widened at base and narrowed at apex, with short, black dorsal spine at tip; dorsal margin near the base with small protuberance bearing two pale setae. Paramere (Fig. 3G, H) with basal part bilobed, distal lobe elongated and narrow, slightly darkened at tip. Aedeagus (Fig. 3G) apically bent downwards, bifid at tip.

Female. Body length 3.8 mm, wing length 4.7 mm, antenna length 0.7 mm (N = 1). General body coloration lighter than male.

Female terminalia (Fig. 3I–J). Yellow. Cercus curved dorsally (Fig. 3I), gradually tapered to pointed tip. Genital fork broad, as long as width, not extending to base of cercus. Hypovalva elongated, blade-shaped reaching slightly before tip of cercus, with distinct dark spot at basal area. Distal end bearing dorsal and ventral serration (Fig. 3J).

Etymology. Specific name "*koreana*" refers to the country of its discovery, Korea.

Distribution. The species is widely distributed in Korea, including Jejudo Island.

Habitats. This species is found along intermittent mountain streams in moist mixed forests with grassy vegetation (Fig. 4A) and in wet deciduous forest along the rocky margins of small mountain streams (Fig. 4B). Adults share their habitats with *D.* (*E.*) *globulithorax* on Mount Bangtaesan and with *D.* (*E.*) *tabashii* on Mount Jirisan.

Period of activity. Adults are mainly active from July through August.

Figure 4. Habitats of *Dicranomyia* (*Erostrata*) *koreana* sp. nov. **A** Jejudo Island **B** Mt. Jirisan.

Remarks. In terms of the shape of the male terminalia, *D.* (*E.*) *koreana* sp. nov. is similar to *D.* (*E.*) *tabashii*, but it can be distinguished by the following characters: palpus 3-segmented (vs 2-segmented); male seventh sternite with weakly darkened, conical internal sac with round entrance (vs strongly darkened, U-shaped internal sac); paramere with darkened tip (vs without). This species is also similar to another species, *D.* (*E.*) *jejuensis* sp. nov. based on the male genital structures, but it can be distinguished by the following characters: male seventh sternite with a deep, conical internal sac (vs shallow, V-shaped notch); gonostylus with two setae from tubercle (vs a single seta); paramere without hook at tip (vs with hook).

The male genitalia of *D.* (*E.*) *koreana* sp. nov. from Mount Bangtaesan differs from other materials of the species based on the shape of the seventh sternite internal sack (shallow conical without rounded mouth) and paramere distal lobe (pointed tip). However, additional specimens are needed to determine whether this difference is due to intra- or interspecific variation.

DNA barcode analysis

The 676-bp *COI* sequences contained 190 variable sites, of which 156 were parsimony-informative. The interspecific divergences (*p*-distances) within subgenus *D.* (*Erostrata*) ranged from 11.54% to 16.42%, with a mean distance of 13.17% across the entire dataset (Table 2), whereas the intraspecific genetic distances ranged from 0% to 0.59%: from 0% to 0.15% in *D.* (*E.*) *jejuensis* sp. nov., 0.59% in *D.* (*E.*) *koreana* sp. nov., and 0.15% in *D.* (*E.*) *tabashii*. The maximum intraspecific genetic distance (0.59%) was much smaller than the minimum interspecific one (11.54%). The NJ tree (Fig. 5) indicated that the monophyly of each of the new species was highly supported, as was that of subgenus *D.* (*Erostrata*) (Fig. 5).

Table 2. Estimates of genetic divergence (%) between sequences. The number of base differences per site from between sequences are shown. Standard errors (%) are shown above the diagonal and were obtained by a bootstrap procedure (1,000 replicates). All positions containing gaps and missing data were eliminated (complete delete option).

	Species	Accession number	1	2	3	4	5	6	7	8	9	10	11
1	*D.* (*E.*) *globithorax*	OM102980	–	1.36	1.36	1.36	1.36	1.37	1.28	1.42	1.41	1.37	1.34
2	*D.* (*E.*) *jejuensis* sp. nov.	OM102981	13.61	–	0	0.15	1.37	1.35	1.36	1.34	1.33	1.33	1.45
3		OM102982	13.61	0	–	0.15	1.37	1.35	1.36	1.34	1.33	1.33	1.45
4		OM102983	13.76	0.15	0.15	–	1.36	1.34	1.36	1.34	1.33	1.34	1.45
5	*D.* (*E.*) *koreana* sp. nov.	OM102979	15.24	14.94	14.94	14.79	–	0.30	1.38	1.21	1.20	1.31	1.45
6		OP081140	15.38	14.64	14.64	14.50	0.59	–	1.40	1.22	1.21	1.31	1.45
7	*D.* (*E.*) *submelas*	OM102978	11.54	14.64	14.64	14.79	15.09	15.68	–	1.43	1.43	1.37	1.36
8	*D.* (*E.*) *tabashii*	OM102975	16.12	14.94	14.94	14.79	11.69	11.83	16.42	–	0.15	1.29	1.44
9		OM102976	15.98	14.79	14.79	14.64	11.54	11.69	16.27	0.15	–	1.30	1.44
10	*D.* (*E.*) *yazuensis*	OM102977	15.24	12.43	12.43	12.57	13.46	13.46	13.76	12.87	13.02	–	1.30
11	*D.* (*D.*) *kandybinae*	OP093621	14.50	16.27	16.27	16.42	16.12	16.12	14.50	15.68	15.53	12.57	–

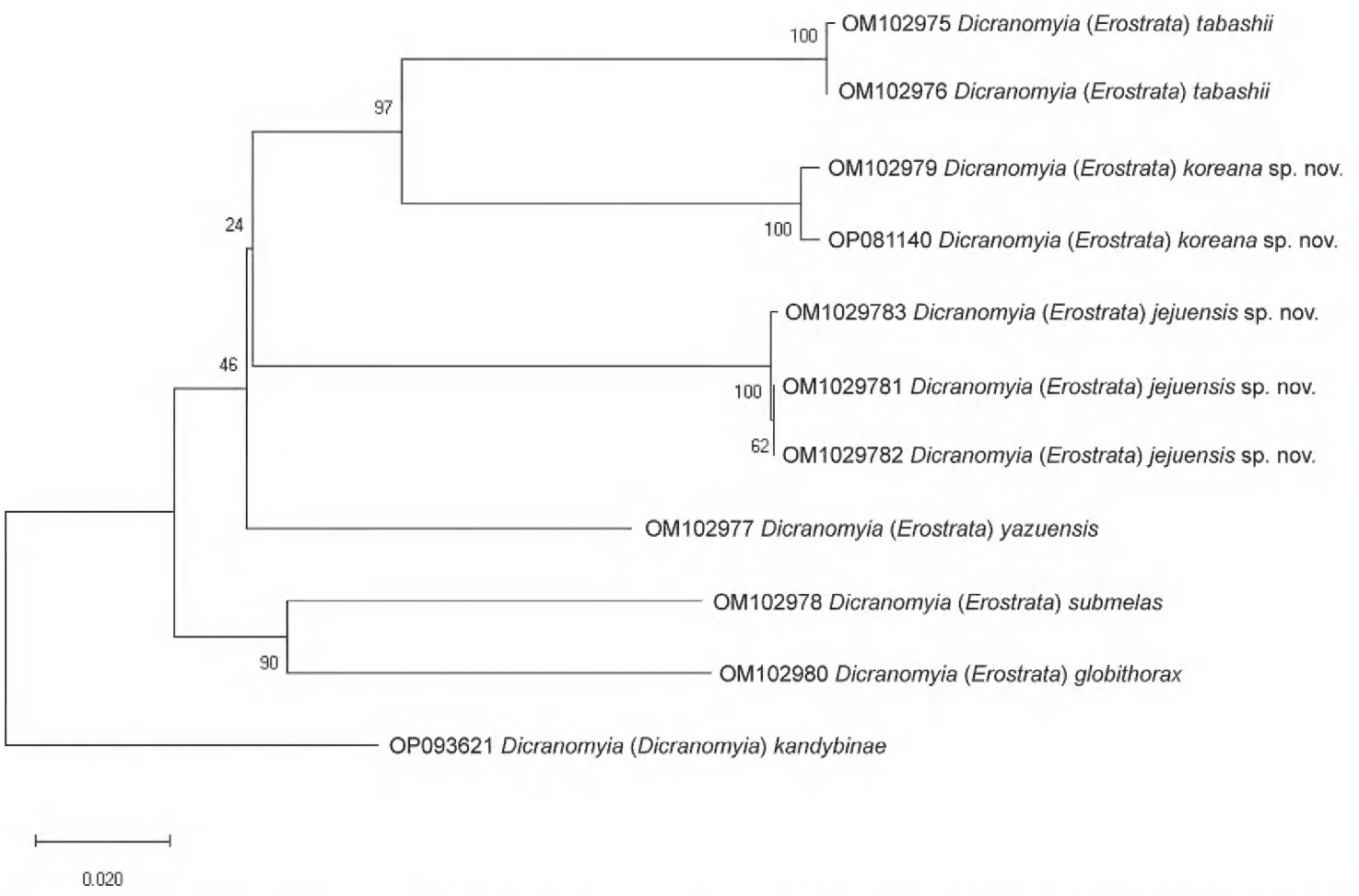

Figure 5. Neighbor-joining (NJ) Kimura-2-parameter tree based on the analysis of the *COI* of six Korean *Dicranomyia* (*Erostrata*) species and *D.* (*Dicranomyia*) *kandybinae* as outgroup. Numbers at the nodes indicate NJ bootstrap support values.

Discussion

This is the first study to use DNA barcoding for the delimitation of the *D.* (*Erostrata*) species. The present study identified two new species using both morphological and molecular data. According to the NJ tree (Fig. 5), the subgenus includes two major clades, which can be distinguished based on the presence or absence of numerous black, strong spines on the mesal face of gonostylus. Indeed, *D.* (*E.*) *jejuensis* sp. nov.,

D. (*E.*) *koreana* sp. nov., *D.* (*E.*) *tabashii*, and *D.* (*E.*) *yazuensis* can be distinguished from other members of their subgenus based on the shape and mesal face (without lots of black, strong spines) of their gonostyli. Two hypotheses may be considered: i) this clade can be classified into morphological species groups, or ii) it can be elevated to a new subgenus. Additional materials are needed to more accurately reconstruct phylogenetic relationships within genus *Dicranomyia*.

Based on our morphological examinations of the materials, we also found that some specimens identified as *D.* (*E.*) *tabashii* by Podenas et al. (2019) are actually specimens of *D.* (*E.*) *koreana* sp. nov. Based on our observation, unknown cryptic species of crane flies could also be detected and identified using molecular data.

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References

Alexander CP (1924) New or little-known crane flies from northern Japan (Tipulidae, Diptera). Philippine Journal of Science 24: 531–611.

Alexander CP (1931a) New or little-known Tipulidae from the Philippines (Diptera). IX. Philippine Journal of Science 45: 415–448.

Alexander CP (1931b) New or little-known Tipulidae from the Philippines (Diptera). X. Philippine Journal of Science 46: 9–38.

Alexander CP (1934) New or little-known Tipulidae from eastern Asia (Diptera). XXI. Philippine Journal of Science 55: 19–60. https://doi.org/10.1093/aesa/27.1.55

Alexander CP (1959) Undescribed species of crane-flies from the Himalaya mountains (Tipulidae, Diptera). IV. Journal of the New York Entomological Society 67: 223–235.

Alexander CP (1967) New or little-known Tipulidae from eastern Asia (Diptera). LX. Philippine Journal of Science 95: 227–266.

Cumming JM, Wood DM (2017) Adult morphology and terminology. In: Kirk-Spriggs AH, Sinclair BJ (Eds) Manual of Afrotropical Diptera. Vol. 1. Introductory chapter and keys to Diptera families. Suricata 4. South African National Biodiversity Institute, Pretoria, 89–133.

de Jong H (2017) Limoniidae and Tipulidae (Crane Flies). In: Kirk-Spriggs AH, Sinclair BJ (Eds) Manual of Afrotropical Diptera. Vol. 2. Nematocerous Diptera and Lower Brachycera. Suricata 5. South African National Biodiversity Institute, Pretoria, 427–477.

Edwards FW (1919) Results of an expedition to Korinchi Peak, Sumatra. Part III. Invertebrates. II. Diptera. Collected in Korinchi, West Sumatra, by Messrs. H. C. Robinson and C. Boden Kloss. Journal of the Federated Malay States Museums 8: 7–59.

Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Molecular Marine Biology and Biotechnology 3(5): 294–299.

Kato D, Tachi T, Gelhaus J (2018) Revision of the subgenus *Dicranomyia* (*Erostrata*) Savchenko, 1976 (Diptera, Limoniidae) of Japan. Zootaxa 4441(1): 181–194. https://doi.org/10.11646/zootaxa.4441.1.11

Kimura M (1980) A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. Journal of Molecular Evolution 16(2): 111–120. https://doi.org/10.1007/BF01731581

Kumar S, Stecher G, Li M, Knyaz C, Tamura K (2018) MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. Molecular Biology and Evolution 35(6): 1547–1549. https://doi.org/10.1093/molbev/msy096

Oosterbroek P (2023) Catalogue of the Craneflies of the World (CCW). https://ccw.naturalis.nl/ [Accessed on 24.03.2023]

Osten Sacken CR (1869) Monographs of the Diptera of North America. Part IV. Smithsonian Miscellaneous Collections 8(219): 1–345.

Podenas S, Seo HY, Kim T, Hur JM, Kim AY, Klein TA, Kim HC, Kang TH, Aukštikalnienė R (2019) *Dicranomyia* crane flies (Diptera: Limoniidae) of Korea. Zootaxa 4595(1): 1–110. https://doi.org/10.11646/zootaxa.4595.1.1

Podenas S, Park S, Byun H, Kim A, Klein TA, Kim H, Aukštikalnienė R (2020) New data on Limoniinae and Limnophilinae crane flies (Diptera: Limoniidae) of Korea. Journal of Species Research 9(4): 492–531. https://doi.org/10.12651/jsr.2020.9.4.492

Rogers JS (1930) The summer crane-fly fauna of the Cumberland Plateau in Tennessee. Occasional Papers of the Museum of Zoology 215: 1–50.

Savchenko EN, Krivolutskaya GO (1976) Limoniid flies (Diptera, Limoniidae) of the South Kuril and South Sakhalin. Akad. Nauk. Ukr. SSR, Kiev, 160 pp. [in Russian]

Simon C, Frati F, Beckenbach A, Crespi B, Liu H, Flook P (1994) Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. Annals of the Entomological Society of America 87(6): 651–701. https://doi.org/10.1093/aesa/87.6.651

Suh KI, Hwang JM, Bae YJ, Kang JH (2019) Comprehensive DNA barcodes for species identification and discovery of cryptic diversity in mayfly larvae from South Korea: Implications for freshwater ecosystem biomonitoring. Entomological Research 49(1): 46–54. https://doi.org/10.1111/1748-5967.12334